International Journal of Bio-Technology and Research (IJBTR)
ISSN(P): 2249-6858; ISSN(E): 2249-796X
Vol. 5, Issue 4, Aug 2015, 19-26

γ -ORYZANOL IMPROVES SERUM AND HEPATIC BIOMARKERS IN STREPTOZOTOCIN-NICOTINAMIDE (STZ-NA) DIABETIC RATS

KOMAL CHAUHAN

National Institute of Food Technology, Entrepreneurship and Management, (NIFTEM)

Kundli, Haryana, India

ABSTRACT

Diabetes mellitus; a clinical syndrome is associated with a large number of lipid abnormalities. Hyperlipidemia and diabetes were induced in male albino wistar strain through dietary and pharmacological means. γ-Oryzanol; was supplemented at100mg/kg.b.w (OZ-100)and at 200 mg/kg b.w. (OZ-200) orally to STZ-NA induced diabetic rats for a period of six weeks. Results showed that γ-oryzanol resulted in marked improvement in serum and hepatic biomarkers of treated animals as compared to untreated ones. γ-Oryzanol strengthened the antioxidant defense system, prevented hepatic damage by scavenging free radicals and reducing oxidative stress thereby exerting a protective role against several metabolic diseases.

KEYWORDS: Oryzanol, Diabetes Mellitus, Antioxidants, Hyperlipidemia, Streptozotocin, Nicotinamde

INTRODUCTION

Diabetes mellitus; a clinical syndrome with variable phenotypic expression; characterized by chronic hyperglycaemia and insulin deficiency. Inadequate insulin secretion adversely affects the lipid metabolism, leading to lipid abnormalities; dyslipidemia, eventually increasing the risk of atherosclerosis and cardiovascular diseases (CVD). According to WHO, 171 million people worldwide are suffering from diabetes mellitus and the number is likely to increase up to 366 million by 2030 (WHO 1999). CVD is prime cause spelling disability and death in diabetics. There is ample evidence indicating that fatty acids, which under normal circumstances act as physiological fuels for the β-cells, become toxic when present at elevated concentrations for prolonged periods of time (McGarry & Dobbins,1999). This results in detrimental effects at cellular and tissue level thereby rendering the diabetics to deleterious clinical manifestations (Godin et al. 1988; Niskanen et al. 1995). The search for appropriate drugs from natural products viz. underutilized parts of plants, fruits and vegetables having dual features of hypoglycaemic and hypolipidemic compounds with no undesirable side effects have been the main focus of research for few decades.

One of the phytochemicals found in high concentration in rice is γ- oryzanol. It is a mixture of sterol esters of ferulic acid and was first isolated in 1954 (Kaneko and Tsuchiya, 1955). The main sterols esterified are cycloartenol and 24-methylene cycloartanol (4, 4`-dimethylsterols) and β- sitisterol and campesterol (4-desmethylsterols). The composition of γ-Oryzanol varies with the rice variety (DeDeckere and Korver, 1996).

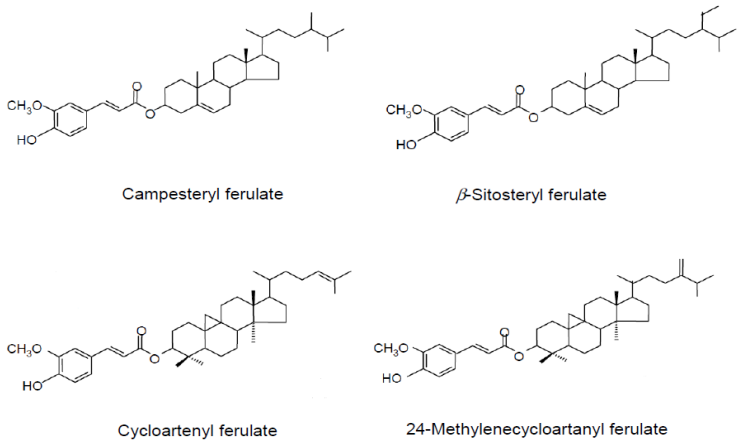

Figure 1: Chemical Structures of Major Components of Γ-Oryzanol

While the health aspects of oryzanol have been studied extensively in the past, there have been few reports on the physiological functions of oryzanol in relation to diabetes. Furthermore, the dose dependent comparative effects of OZ on the lipid metabolism and antioxidative status remain unclear. Thus, this study was conducted to evaluate and compare the effects of dietary feeding of γ-Oryzanol on the lipid metabolism and antioxidant enzyme activity in STZ-NA induced diabetes in rats.

The study protocol was approved by Institutional Animal Ethics Committee (IAEC) of the University constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Healthy male albino wistar rats of 6 weeks age (50.2±2.86g) were procured from the small animal house of Chaudhary Charan Singh Haryana Agriculture University Hissar (CCSHAU), India

Streptozotocin (STZ), Glibenclamide (GLM); Nicotinamide (NA) were obtained from Sigma–Aldrich, St. Louis, USA. γ- oryzanol was purchased from Qingdao Reach International Inc, China.

Rats were rendered diabetic by single intraperitoneal injection of freshly prepared streptozotocin (60mg kg^{-1}) in 0.1 M citrate buffer (pH 4.5) in a volume of 1ml kg^{-1} body weight (Siddique et al., 1987). Nicotinamide (NA) was dissolved in normal saline and administered (120 mg/kg, i.p) 15 min before STZ (Masiello et al., 1998). The blood glucose levels of rats within a range of 200-300 mg/dl were considered to be diabetic and were selected for the study

Animals with similar average body weight were randomly divided into five groups of six animals each as under: Group I: Normal control, fed normal fat diet; Group II: Diabetic control fed high fat high cholesterol diet (HFHC) (15%coconut oil; 1%cholesterol and 0.125% taurocholate); Group III: DC-OZ-100; HFHC+ γ-Oryzanol (OZ) (100mg/kg body weight). Group IV: DC-OZ-200; fed HFHC+ γ-Oryzanol (OZ) (200mg/kg body weight); Group V: DC-GLM; fed HFHC and administered glibenclamide (GLM) (5mg/kg body weight i.p. in 10% DMSO). Basal (20% protein, 5% protein, 5% fat and 5% fibre, 60% carbohydrates and 10% mixture of vitamins and minerals.) and experimental diets were isoenergic (~3600C) and were freshly prepared weekly in a pathogen free sterilized room (Reeves et al; 1993) and were stored at -20^0C. The dietary schedule was followed for six weeks and the animals after an overnight fast were bled from retrorbital plexus for evaluation of biochemical parameters; thereafter, animals were sacrificed by cervical decapitation.

Enzyme assay involved, hexokinase activity (Brandstup et al., 1957), glucose-6-phosphate dehydrogenase (G-6PD) (Baginsky et al., 1974), HMG Co.A reductase activity was assayed by the method of Rao and Ramakrishnan (1975) and expressed as the ratio of absorbance of HMG- CoA to mevalonate. Lipoprotein lipase (LPL) was measured by Korn method (1955), lecithin cholesterol acyly transferase (LCAT) was measured using Hitz et al., (1983), lipid peroxidation, (TBARS) (Ohkawa et al., 1979), reduced glutathione (GSH) (Sedlak & Lindsey, 1968) Serum glutamate pyruvate transaminases (SGPT) and serum glutamate oxaloacetate transaminases (SGOT) were measured spectrophotometrically by utilizing the method of Reitman and Frankel (1979). Acid (ACP) and alkaline phosphates (ALP) were estimated using King and Armstrong method (1934).Gamma glutamyl transferase (GGT) activity was measured by the Rosalki and Rau Method (1972). Insulin level was assayed by Enzyme Linked Immunosorbant Assay (ELISA) kit (Anderson et al., 1993); liver glycogen (Montgomery, 1957) and protein content using Bio-Rad protein assay kit and BSA as standard.

Results are expressed as Mean ± Standard Error of Mean (SEM) of 6 rats. Statistical analysis involved Analysis of Variance (ANOVA- one-way). Tukey's post-hoc multiple comparison test was carried out using SPSS (version 16.0) and student's 't'-test using Sigma Plot (version 8.0). The values at $p \leq 0.05$ were considered as statistically significant.

Table 1 depicts that supplementation of high fat diet to STZ-NA diabetic rats resulted in significant decrease ($p \leq 0.05$) in liver weight, glycogen; insulin levels and hexokinase activity, with a significant increase in glucose -6-phosphate dehydrogenase (G-6PD) activity as compared to normal control (Group-I). OZ administrated rats tended to nullify the effect and restored the levels to near normal. The diabetogenic effect may be due to increased gycogenolysis; gluconeogenesis or due to the irreversible destruction of β cells of islets of langerhans by STZ resulting in insufficient secretion of insulin. The insulinotropic action of OZ probably may be due to stimulation of β cells thus restoring their function and showing a protective efect. Glibenclamide, acts in a similar manner and potentiates the release of insulin by pancreatic cells which has been confirmed in previous studies (Gerich, 1989). Restored glycogen levels are indicative of antiglycaemic effect of the supplemented diets as glycogenesis involve restoration of the insulin levels which have been confirmed in previous studies (Chauhan et al., 2015; Chauhan et al., 2012;Cheng et al., 2010; Sharma and Garg, 2009). Hexokinase activity improved remarkably with an appreciable reduction in G6PD activity in OZ treated rats probably by its insulinotropic action thereby reducing glucose absorption, increased uptake of glucose by peripheral tissues and preventing glycogenolysis and gluconeogenesis. The results are in accordance with previous studies (Thangapandiyan and Miltonprabu, 2014).

Lipid marker enzymes (lipoprotein lipase (LPL); HMG CoA reductase and lecithin cholesterol acyl transferase (LCAT)) considerably decreased in diabetic control group implying altered lipid metabolism (Table 1). Coadministration of OZ had a positive effect and resulted in increased activities of lipid metabolizing enzymes. LPL regulates triglyceride metabolism. Hyperlipidemia associated oxidative stress in diabetics result in constant build up of free radicals and reactive oxygen species which inhibits the enzymatic activity further aggravating hypertriglyceridemia. HMG CoA reductase is the rate limiting enzyme in cholesterol biosynthesis (Goldstein and Brown, 1990). The HMG-CoA/mevalonate ratio is inversely proportional to the enzyme activity. Reduction in the enymic activity of treated animals indicates decreased cholesterol biosynthesis and increased catabolism through bile acid secretion, consistent with previous reports (Chauhan et al., 2015). LCAT controls HDL-C metabolism; esterifying free cholesterol into cholesteryl esters in HDL, henceforth HDL reduces the amount of deposited cholesterol in endothelium by retrieving cholesterol from peripheral cells and other

lipoproteins to the liver for excretion in bile, thereby reducing the risk of CVD. (Wang et al., 2014; Zou et al., 2005; Martinez et al., 2004). Owing to its unsaponifiable nature and antioxidative potential, OZ inhibits accumulation of TG, decreases absorption of dietary cholesterol or increases bile acids or fecal sterols excretion subsequently leading to increased lipid marker enzyme activities. The results are in accordance with earlier study which demonstrated that rice bran oil and its components improves insulin resistance and lipid metabolism (Chou et al., 2009)

Table 1: Effect of Γ -Oryzanol (OZ) on Liver Weight, Insulin, Glycogen and Biochemical Parameters in STZ-NA Induced Diabetic Rats

Treatment	Liver Weight (G)	Insulin (G/Dl)	Glycogen (Mg/G Wet Tissue)	Hexokinase (U/Mg / Protein/Min)	G6PD (U/Mg Protein/ Min)	LPL Mmoles of Glycerol Liberated /Ml	HMG Co. A Reductase/ Mevalonate Ratio	LCAT Mmoles of Cholesterol Esterified/ Ml
Group-I	23.4±1.21	16.9±0.31	45.4±0.21	9.1±0.85	0.22±0.01	7.7±0.45	1.57±0.01	67.4±2.21
Group-II	20.3±1.93[a]	3.7 ±0.28[a]	24.4±0.34[a]	4.7±0.47[a]	0.49±0.02[a]	5.1±0.23[a]	0.83±0.02[a]	51.6±2.15[a]
Group-III	22.7±1.25[bc]	15.3 ±0.24[b]	41.6 ±0.14[bc]	5.2±0.61[bc]	0.37±0.01[bc]	5.8±0.27[NS]	1.43±0.01[bc]	58.4±2.34[bc]
Group-IV	24.1 ±2.13[b]	15.8 ±0.26[b]	61.2 ±0.26[b]	7.3±0.72[bc]	0.28±0.01[bc]	6.6±0.36[b]	1.49±0.01[b]	62.3±2.14[bc]
Group-V	24.2 ±2.12[b]	15.8 ±0.28[b]	60.5 ±0.29[b]	6.8±0.62[b]	0.23±0.01[b]	6.7±0.21[b]	1.51±0.01[b]	63.8±2.54[b]

Values are Mean ± SEM of 6 rats in each group
[a]p≤0.05 : Significantly different from Group-I
[b]p≤0.05 : Significantly different from Group-II
[c]p≤0.05 : Significantly different from Group-V
NS: Non Significant

Hyperlipidemia and STZ induces oxidative stress as reflected by increased TBARS and decreased GSH levels in diabetic control group (Table). Furthermore, oxidative stress leads to hepatic damage; dysfunction; disrupting the membrane integrity resulting in leakage of enzymes in circulation. A marked elevation in hepatic enzymatic activities (ALT; AST; ACP; ALP and GGT) of high fat and high cholesterol fed diabetic rats is indicative of hepatocellular injury (Table 2). OZ enriched diets resulted in substantial lowering in TBARS levels with concomitant increase in GSH content and marked improvement in hepatic biomarkers thus protecting the tissue from oxidative assault. Restoration of enzymic activities, perooxidative potential and glutathione in OZ treated rats were almost comparable to glibenclamide indicating, OZ can be used to ameliorate hyperlipidemia induced oxidative stress in diabetics probably due to its unsaponifiable nature and by scavenging the free radicals.

Table 2: Effect of Γ -Oryzanol (OZ) on Hepatic Oxidative Biomarkers in STZ-NA Induced Diabetic Rats

Treatment	TBARS (Nm Oftbars/Mg Protein)	GSH (Mg/G Tissue)	ALP (U/L)	ACP (U/L)	AST* (IU/L)	ALT* (IU/L)	GGT# (IU/L)
Group-I	41.2±6.3	16.5±1.21	60.4±1.04	39.8±1.13	73.5±2.13	25.2±1.12	13.9±2.13
Group-II	1392.2±4.2[a]	11.2± 1.83[a]	78.6±1.23[a]	76.1±1.65[a]	121.4±2.15[a]	60.1± 1.17[a]	24.8±2.16[a]
Group-III	843.2 ±4.9[bc]	29.8 ±1.75[bc]	68.5±2.16[bc]	52.1±1.57[bc]	81.5± 2.34[b]	28.3 ±1.34[b]	17.1±1.43[bc]
Group-IV	827.6 ±4.8[bc]	36.7± 1.84[bc]	64.3±2.17[bc]	43.7±1.31[b]	81.1 ±2.14[b]	29.0 ±1.17[b]	14.3±1.23[bc]
Group-V	1012.5 ±4.8[b]	34.3± 2.16[bc]	62.1±1.04[b]	42.6±1.21[b]	79.3±2.43[b]	29.6±1.23[b]	15.6±1.12[bc]

Values are Mean ± SEM of 6 rats in each group
*µmol of pyruvate liberated per hour
#µmol of p-nitroanilidine liberated per minute
[a]p≤0.05 : Significantly different from Group-I
[b]p≤0.05 : Significantly different from Group-II
[c]p≤0.05 : Significantly different from Group-V

CONCLUSIONS

The study suggest that γ-Oryzanol for its antioxidative, antidiabetic and hypolipidemic potential can be supplemented to diets of vulnerable section of population to delay or prevent the onset and progression of diabetes and its complications.

ACKNOWLEDGMENTS

The authors like to acknowledge University Grant Commission for financial support and Dr. Ajit Kumar, Vice Chancellor, NIF TEM for his unstinting support and valuable suggestions

REFERENCES

1. Anderson, L., Dinesen, B., Jorgonsen, P.N., Poulsen, F., Roder, M.E. (1993). Enzyme immune assay for intact human insulin in serum or plasma. Clinical Chemistry, 39, 578-582.
2. Baginsky, E.S., Foa, P.P.,& Zad, B. (1974) Glucose-6-phosphatase. In: Bergemeyer HU, editor. Methods of enzymatic analysis. 2nd Ed. New York: Academic press. 788–792.
3. Brandstup, N., Kirk, J.E., & Bruni, C. (1957) Determination of hexokinase in tissues. Journal of Gerontology. 12:166–171.
4. Chauhan K., Chauhan, B.,& Bajaj, G. (2015). Effects of rice bran oil and gamma-γ-Oryzanol on antioxidant defense in streptozotocin nicotinamide induced diabetic rats. International Journal of Engineering Sciences & Research Technology, 4(6), 458-471.
5. Chauhan, K., Sharma, S., Rohatagi, K.,& Chauhan, B. (2012). Antihyperlipidemic and antioxidative efficacy of Catharanthus roseus Linn (Sadabahar) in Streptozotocin induced diabetic rats, Asian Journal of Pharmaceutical and Health Sciences. 2 (1), 235-243.
6. Cheng, H.H, Ma, C.Y., Chou, T.W., Chen, Y.Y., & Lai, M.H. (2010).Gamma-γ-Oryzanol ameliorates insulin resistance and hyperlipidemia in rats with streptozotocin/nicotinamide-induced type 2 diabetes. International Journal of Vitaminology Nutrient Research, 80(1), 45-53.
7. Chou, T.W., Ma, C.Y., Cheng, H.H., Chen, Y.Y., & Lai, M.H. (2009) A rice bran oil diet improves lipid abnormalities and suppress hyperinsulinemic responses in rats with streptozotocin/nicotinamide-induced type 2 diabetes, Journal of Clinical Biochemistry and Nutrition. 45, 29–32.
8. DeDeckere, E.A.M., & Korver, O. (1996).Minor constituents of rice bran oil as functional foods. Nutrition Review, 54(11), S120-S126.
9. Gerich, J.E. (1989) Oral hypoglycemic agents. New Eng Journal Medicine. 321, 1231-1245.
10. Godin, D. V., Wohaieb, S. A., Garnett, M. E. & Goumeniouk, A. D. (1988). Antioxidant enzyme alterations in experimental and clinical diabetes. Mol Cell Biochem 84 (2), 223-231.
11. Goldstein ,J.L., & Brown, M.S. (1990) Regulation of the mevalonate pathway. Nature, 343, 425–430.
12. Hitz, J., Steinmetz, J.,& Siest, G. (1983) Plasma lecithin: cholesterol acyltransferase- reference values and effects

of xenobiotics. Clin Chim Acta 133,85–96.

13. Kaneko, R., Tsuchiya, T. (1995). New compound in rice bran and germ oils. Chem Abstr 49, 4897b.
14. King, E.J., & Armstrong,A.R. (1934). Determination of serum and bile phosphatase activity. Cannadian Medical Association Journal, 31, 56-63.
15. Korn, E.D. (1955) Clearing factor: a heparin activated lipoprotein li-pase: isolation and characterization of enzyme from normal rats. Journal Biology Chemistry 215, 1–26.
16. Martinez, L.O., Jacquet, S. Terce, F., Collet, X., Perret, B., & Barbaras, R. (2004). New insight on the molecular mechanisms of highdensity lipoprotein cellular interaction. Cell Molecular Life Science, 61, 2343–2360.
17. Masiello, P., Broca, C., Gross, R., Roye, M., Manteghetti, M., Hillaire-Buys, D., Novelli, M., & Ribes, G. (1998). Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes, 47, 224–229.
18. McGarry, J.D, & Dobbins, R.L. (1999). Fatty acids, lipotoxicity and insulin secretion. Diabetologia, 42,128 –138.
19. Montgomery, R. (1957). Determination of glycogen. Archives Biochemistry Biophysics, 67,378-386.
20. Niskanen, L. K., Salonen, J. T., Nyyssönen, K. & Uusitupa. M. I. (1995). Plasma lipid peroxidation and hyperglycaemia: a connection through hyperinsulinaemia? Diabet Med 12 (9),802-808.
21. Ohkawa, H., Oshishi, N. & Yagi, K. (1979). Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Annals of Biochemistry, 95, 351-358.
22. Rao, A. V & S. Ramakrishnan. (1975). Indirect assessment of hydroxymethylglutaryl CoA reductase (NADPH) activity in liver tissue. Clinical Chemistry, 21, 1523-1525.
23. Reeves, P.G., Nielsen, F.H., Fahey, G.C. Jr. (1993) AIN-93 Purified diets for laboratory rodents: Final report of the American institute of nutrition adhoc writing committee on the reformulation of the AIN-76 a rodent diet. Journal of Nutrition, 123: 1939-1951.
24. Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal Clinical Pathology, 28, 56-63.
25. Rosalki, S.B., Rau, D. (1972). Serum γ-glutamyl transpeptidase activity in alcoholism. Clinical Chemistry Acta, 39, 41-47.
26. Sedlak, J., & Lindsey, R.H. (1968). Estimation of total protein bound and non protein sulphydral groups in tissues with Ellman's reagent. Annals of Biochemistry, 25, 192.
27. Sharma, N. & Garg, V. (2009). Antidiabetic and antioxidant potential of ethanolic extract of Butea monosperma leaves in alloxan induced diabetic mice, Indian Journal of Biochemistry and Biophysics, 46, 99 – 105.
28. Siddique, O., Sun,Y., Lin, J.C., & Chien, Y.W. (1987). Facilitated transdermal transport of insulin. Journal of Pharmaceutical Science, 76, 341- 345.
29. Thangapandiyan, S., & Miltonprabu , S. (2014).Ameliorative effect of epigallocatechin gallate on sodium fluoride

induced oxidative stress mediated metabolism in rat. International Journal of Pharmacology and Toxicology, 2(2), 76-85.

30. Wang, Y., Li, Y., Sun, A., Wang, F., & Yu, G. (2014). Hypolipidemic and antioxidative effects of aqueous enzymatic extract from rice bran in rats fed a high-fat and -cholesterol diet. Nutrients, 6, 3696-3710.
31. WHO. 1999. Report of WHO/IDF Consultation: Definition, Diagnosis and classification of Diabetes mellitus and its complications. Retrieved 2010 August from WHO. http://www.staff.ncl.ac.uk/philip.home/ who_dmg.pdf.
32. Zou, Y.P., Lu, Y.H., & Wei, D.Z. (2005).Hypocholesterolemic effects of a flavonoid-rich extract of *Hypericum perforatum*L. In rats fed a cholesterol-rich diet. Journal of Agricultural Food Chemistry, 53, 2462–2466.